

## REPRODUCTION AND DEVELOPMENT OF THE HERMAPHRODITIC SEA-STAR, *ASTERINA MINOR* HAYASHI

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A number of papers have been published on the development of sea-stars belonging to the genus *Asterina*, but the entire process of the development from eggs to juveniles is known only in four species: *A. gibbosa* (Ludwig, 1882; Goto, 1898; MacBride, 1896), *A. burtoni* (James, 1972), *A. coronata japonica* (Komatsu, 1975) and *A. batheri* (Kano and Komatsu, 1978). Among these, description of *A. burtoni* is quite brief and information on the development of other asterinids is fragmental.

Sea-stars generally are gonochoric, but a few hermaphroditic asterinid species, namely *A. batheri*, *A. gibbosa*, *A. scobinata* and *A. pancerii* (Ohshima, 1929; Delavault, 1966; Dartnall, 1970) have been known. The details of breeding in these hermaphroditic species, however, are not well documented, although the gonadal structure and development of some species like *A. gibbosa* (MacBride, 1896) and *A. batheri* (Ohshima, 1929; Kano and Komatsu, 1978) have been reported in detail.

The present study was initiated to determine the breeding and development of *A. minor*, which was recently described as a new species (Hayashi, 1974). The preliminary observations revealed that the present species had a distinct breeding behavior. Moreover, it is found that *A. minor* is hermaphroditic and is able to self-fertilize. This feature being unique among sea-stars, it was felt worthwhile to have a thorough understanding of the breeding and development of this species.

The present paper describes the structure of the gonad, breeding behavior, and development throughout metamorphosis in *A. minor*. It also incorporates the observations on isolated cultures studied during the breeding season.

### MATERIALS AND METHODS

Specimens of *A. minor* were collected from the undersurface of stones at the intertidal zone of Kushimoto, Wakayama Pref., Japan, on several occasions during May of 1972 to June of 1977. They were brought to the laboratory of Toyama University and kept either in groups or individually.

The development of the species from fertilization to the completion of metamorphosis was observed with the help of a dissecting microscope and an inverted microscope. Measurements of living embryos were executed with an ocular micrometer. Examination of the skeletal system was performed on alcohol-fixed specimens treated with potassium hydroxide solution.

For microscopic observation of the reproductive organs, fresh specimens were measured and weighed, and then fixed in Bouin's solution. The fixed material was

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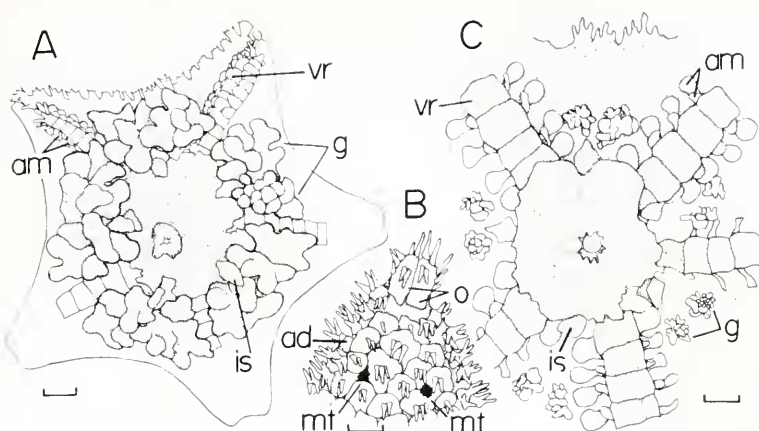


FIGURE 1. A) Aboral view of the arrangement of mature gonads in the specimen, collected on April 25, 1975, just before spawning. Aboral body wall and viscera were removed. am, ampulla; g, gonad; is, interradial septum; vr, vertebral ridge; Scale = 1 mm. B) Remnants of germinal substance, mostly egg fragments, attached to the gonopores in the specimen, collected on June 13, 1974, which was after spawning. ad, adambulacral plate; mt, remnants of germinal substance; o, oral plate. Scale = 500  $\mu$ m. C) Aboral view of the gonads in the specimen collected on June 13, 1974, which was after spawning. am, ampulla; g, gonad; is, interradial septum; vr, vertebral ridge. Scale = 500  $\mu$ m.

serially sectioned at 7  $\mu$ m by routine paraffin method and stained with Delafield's hematoxylin and eosin.

## OBSERVATIONS AND RESULTS

### *Breeding season*

In the present study, no observations were made to confirm spawning in the field. However, it is felt that a possible breeding season of *A. minor* is during May at Kushimoto. This prediction is based on the following circumstantial evidence. In the specimens examined, the gonads were largest in size and nearly mature in late April (Figs. 1A, 2A-F). In adults collected in June, some particles, possibly fragments of ova, were often observed near the gonopores (Fig. 1B). Similar particles were usually observed in the specimens spawned under laboratory conditions. The gonads of the individuals collected in June showed an atrophy and contained degenerating ova (Figs. 1C, 3A, 3B). Spawning in the laboratory as observed for 3 years occurs in the month of May (Table I). The juveniles of this species, each bearing two pairs of tube-feet in each arm, were collected in the month of June. They were estimated to be 20 to 30 days old since their fertilization. In the later months (July onward), the juveniles found in the field were larger than those collected in June (Table II).

### *Structure of the gonad*

A pair of gonads lies at each interradial portion and each gonad is composed of cluster of tubules (Figs. 1A, 1C). Histological observations on the specimens

TABLE I

*Occurrence of spawning of specimens of Asterina minor in the laboratory.*

Number of animals kept together	Date of collection in the field	Time and date of the commencement of spawning in the laboratory
40	April 25, 26, 1974	21:20 May 8, 1974
2	April 25, 1975	17:05 May 2, 1975
10	April 25, 1975	13:00 May 11, 1975
4	April 25, 1975	9:20 May 24, 1975
2	Feb. 16, 1976	20:25 May 12, 1976
2	April 28, 1976	17:30 May 19, 1976
2	April 28, 1976	20:55 May 22, 1976
2	April 28, 1976	0:00 May 25, 1976
2	April 28, 1976	20:00 May 25, 1976

measuring 2.5 to 6.0 mm in R show that this species is a spatial hermaphrodite. Each gonad consists of both the ovarian tubules and the testicular ones (Figs. 2A, 2B) and has a common gonoduct which opens on the oral side of the disk (Figs. 3A, 3B). In the breeding season, the ovarian tubules show pale yellow color and the testicular tubules are whitish and semi-transparent. In general, the testicular tubules lie near the gonoduct and are smaller than the ovarian tubules located in the peripheral region (Fig. 2C). The distribution of the ovarian and testicular tubules, however, varies in different gonads and also in different individuals. In some cases, both sex elements exist in a single tubule (Figs. 2D, 2F). After spawning, all gonads become shrunken and transparent and show a green tint (Figs. 1C, 3A, 3B).

### *Breeding behavior*

Breeding assemblage was observed in the laboratory every year. Although spawning was not observed in the field, assemblage of this species was often found

TABLE II

*Number and size distribution of juveniles (under 17 pairs of tube-feet) collected in the field.*

Number of tube-feet in the longest arm (in pairs)	June 10-12, 1975	July 3-6, 1974	Sept. 11-13, 1973	Feb. 16, 1976
2	10	4		
3		31	1	
4		27	5	
5		6	26	
6			32	
7			42	
8			29	
9		1	7	1
10		1	3	
11		4	5	
12		22	19	
13		36	9	
14		18	3	
15		6	2	
16		3	1	
17		2	1	

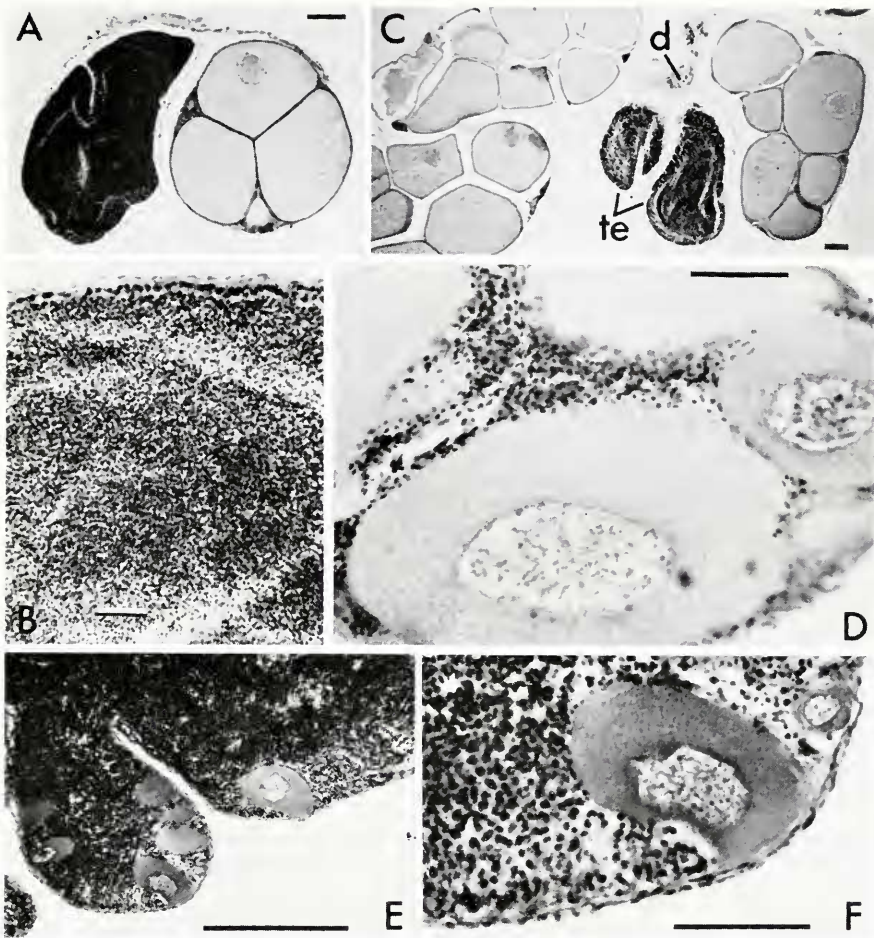


FIGURE 2. Section of the gonad of a specimen of *Asterina minor*, which was fixed just before spawning. Hematoxylin-eosin stain. A) A testicular and an ovarian tubule, containing mature sperms and ova, respectively. B) Magnified picture of the testicular tubule in Figure 2A. C) Section of gonad showing that testicular tubules (te) are situated close to the gonoduct (d). D) A hermaphroditic gonad, in which ovarian part is dominating. E) A hermaphroditic gonad, in which testicular part is dominating. F) Magnified picture of a part of Figure 2E. Scale = 100  $\mu$ m in A, C, E. Scale = 50  $\mu$ m in B, D, F.

in the field in May. The following is a description of the typical process of breeding assemblage.

Forty animals which were collected on April 25 and 26, 1974, began to organize into two groups in a large glass container in the laboratory several days after their collection. These groups were not very stable and animals moved frequently from one group to the other. Generally after about 10 days the assemblages became stable, and no animals seemed to move from their respective group. At this time animals in either group clung to one another along the margins of their bodies, or they were imbricated with others to some extent.



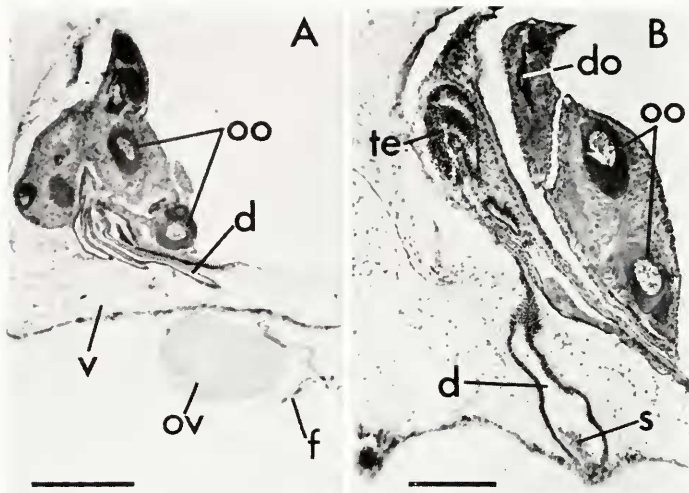


FIGURE 3. Sagittal section of the gonad of a specimen of *Asterina minor*, showing gonoduct and gonopore. A) This specimen was fixed during spawning. Note a spawned egg (ov) close to the body surface of the adult and fertilization membrane (f). d, gonoduct; oo, ovarian ova; v, ventral body wall of the adult. Scale = 50  $\mu$ m. B) This specimen was fixed just after spawning was finished. Note a remnant of sperms (s) in the gonoduct (d) and degenerating ova (do). oo, ovarian ova; te, testis. Scale = 25  $\mu$ m.

At 21:20 May 8, one animal in an assemblage comprised of 25 individuals began to spawn (a, in Fig. 4). As will be described later the eggs got attached to the substratum after their release. About 15 min thereafter, about 80 eggs were laid by this animal. At 22:50, three other animals (b, c, d, in Fig. 4) began to spawn and several minutes later each animal had delivered about 20 eggs. About 30 min thereafter, four more animals (e, f, g, h, in Fig. 4) began to spawn. Two

TABLE III

*Spawning of specimens of Asterina minor in isolated culture.*

Size of animal (mm) R      r	Fresh weight of newly collected animal (mg)	Fresh weight of animal after spawning (mg)	Decrease of weight by spawning (%)	Number of eggs spawned	Term in isolated culture (day)
7.0 4.2	188.3	106.0	44	297	27
6.5 4.5	149.4	100.4	33	233	87
5.6 3.3	148.3	—	—	180	85
5.5 4.1	94.1	77.7	17	243	21
5.4 4.0	196.4	92.8	53	261	21
5.1 3.7	79.0	69.7	12	127	19
5.1 3.3	62.3	45.2	27	132	29
5.0 3.8	89.3	69.8	22	137	39
5.0 3.2	67.6	56.3	17	116	28
4.7 3.5	37.8	29.5	22	87	21
4.2 3.8	45.8	34.9	24	44	100
4.1 2.9	16.4	10.5	36	6	33

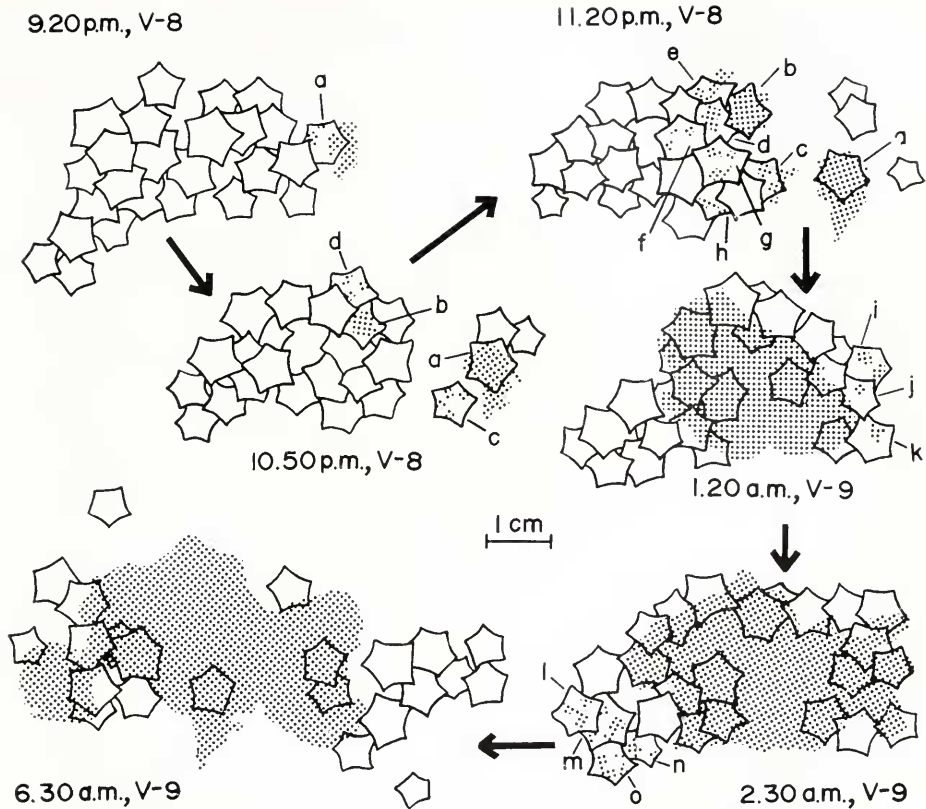


FIGURE 4. Sequential change of the distribution of individuals in a breeding assemblage. Dotted areas show the deposition of eggs. See text for detail.

hours later, three more animals (i, j, k, in Fig. 4) were found to be spawning, and soon four other animals (l, m, n, o, in Fig. 4) followed. After that, the majority of animals in this assemblage were found to be spawning. About 6 hr after the first spawning, all animals seemed to have completed spawning. At 6:30 the following morning (May 9), the assemblage was almost disordered. In the other assemblage, spawning commenced 3 hr after the onset of the spawning in the first one. The process of spawning in the other assemblage was very similar to that in the first. In the evening of May 9, the assemblages were completely disorganized and constituent individuals in either assemblage got mixed. After the disorganization of the assemblages, two egg masses were found, and these were not protected by the adults.

#### *Isolated culture, a proof of self-fertilization*

From the characteristic breeding assemblage observed, it seems that this species engages in cross-fertilization. However, there is a possibility that the species also indulges in self-fertilization, since histological observations show

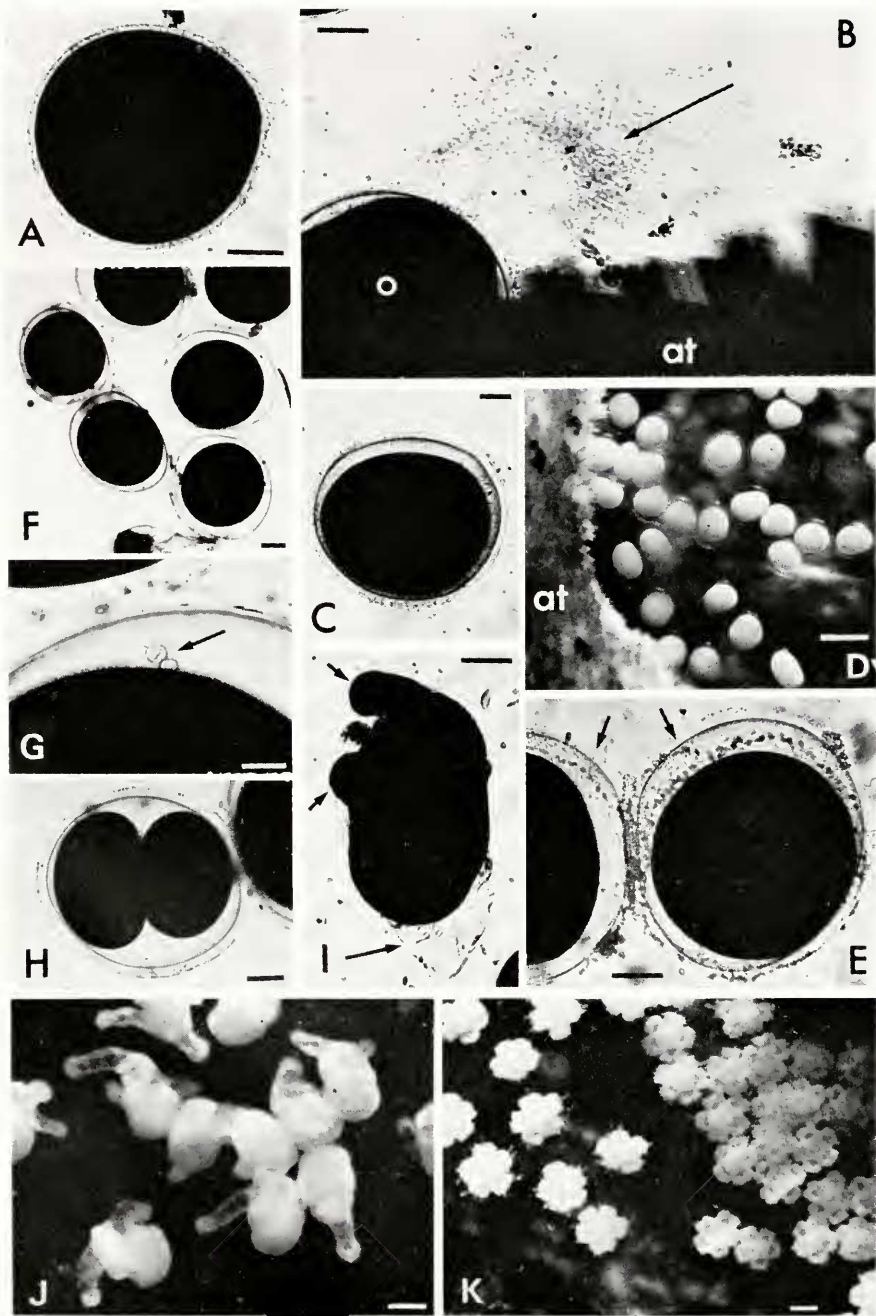


FIGURE 5. Development of specimens of *Asterina minor*. All pictures show living specimens. A) Egg just after spawning. Fertilization membrane was not yet formed. Sperms are crowding around the jelly layer. B) A mass of sperm (arrow) is seen near the spawning

both male and female elements simultaneously maturing in one individual. In order to know whether the species was able to self-fertilize, observations were made on individuals in isolated culture. Specimens, immediately after their collection in the field, were kept individually in small glass jars. Temperature during the culturing was maintained similar to that of the natural habitat, 15 to 25° C. The majority of animals in the isolated culture deposited eggs and these eggs were fertilized with the sperms ejected from the same animal in the absence of any artificial treatment. The details in respect of isolated culture and self-fertilization are given in Table III.

The eggs possibly became mature during the travel through the gonoduct or else as soon as they were released. The number of eggs spawned by one adult is related to the size of the adult. The self-fertilized eggs developed into normal embryos and became normal juveniles through metamorphosis (Fig. 5K). No differences were observed in the fertilization rates or developmental processes in the specimens held in mass culture and those kept in an isolated culture. Adults were collected in different months of the year and kept separately, but they spawned within a limited span of time in the laboratory.

The above observations demonstrate that *A. minor* has an ability to self-fertilize when kept singly. However, it is not clear whether or not self-fertilization occurs when the animal is spawning in the breeding assemblage.

### Development

In this species no fertilization membrane was observed in the freshly laid eggs (Fig. 5A).

The egg is spherical with a diameter of  $437 \pm 5 \mu\text{m}$  (mean  $\pm$  s.e.,  $n = 37$ ). They are transparent yellow and have a jelly layer of about  $20 \mu\text{m}$  thick. A few minutes after spawning, sperm masses are released from the same adult (Fig. 5B) and the sperms are dispersed. The head of the sperm is spherical, about  $3 \mu\text{m}$  in diameter, and the tail is about  $30 \mu\text{m}$  in length. The eggs a few minutes after spawning, attach to the substratum and the neighbouring eggs by their sticky jelly layer. Ten minutes after spawning, elevation of the fertilization membrane is recognized. Figure 5C shows fertilization membrane in an egg 30 min after spawning. The spawned eggs are laid on the bottom in one layer (Figs. 5D, 5E). About 2 hr after spawning, polar bodies are seen in the perivitelline space, which is about  $50 \mu\text{m}$  in height (Figs. 5F, 5G). Three hours after spawning at 20 to 23° C, the first cleavage occurs through the animal-vegetal axis (Figs. 5H, 6A), and 40 min thereafter it is followed by the second cleavage which is perpendicular

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adult (at). Fertilization membrane is being formed in an egg (o), about 10 min after spawning. C) Egg about 30 min after spawning. D) Adult (at) and eggs spawned from it, 1 hr after the commencement of spawning. E) Magnified picture of a part of Figure 5D. Two eggs are attached to each other by their jelly layers (arrows), which lie outside of the fertilization membrane. F) Eggs 2 hr after spawning. G) Magnified view of F. Note polar bodies (arrow). H) First cleavage, 3 hr after spawning. I) Hatching brachiolaria, view from the ventro-lateral (left) side. Long and short arrows indicate fertilization membrane and brachiolar arms, respectively. J) Developed brachiolariae, creeping on the substratum. K) Metamorphosed juveniles, 15 days after spawning. Scale =  $50 \mu\text{m}$  in D, G. Scale =  $100 \mu\text{m}$  in A, B, C, E, F, H, I. Scale =  $200 \mu\text{m}$  in J. Scale =  $100 \mu\text{m}$  in K.



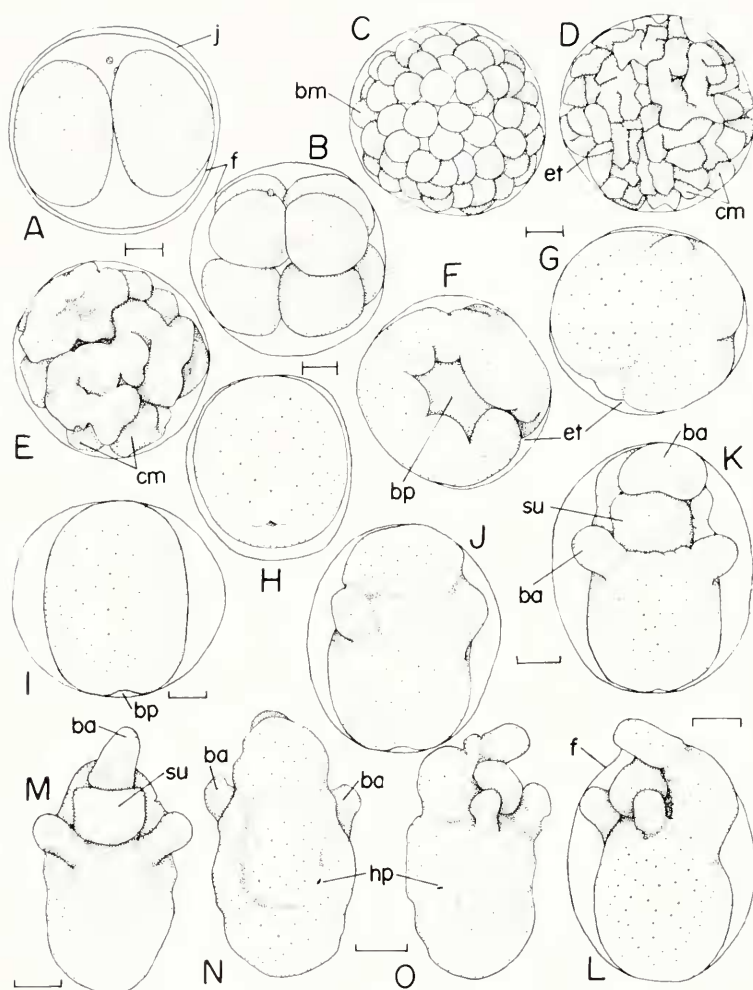


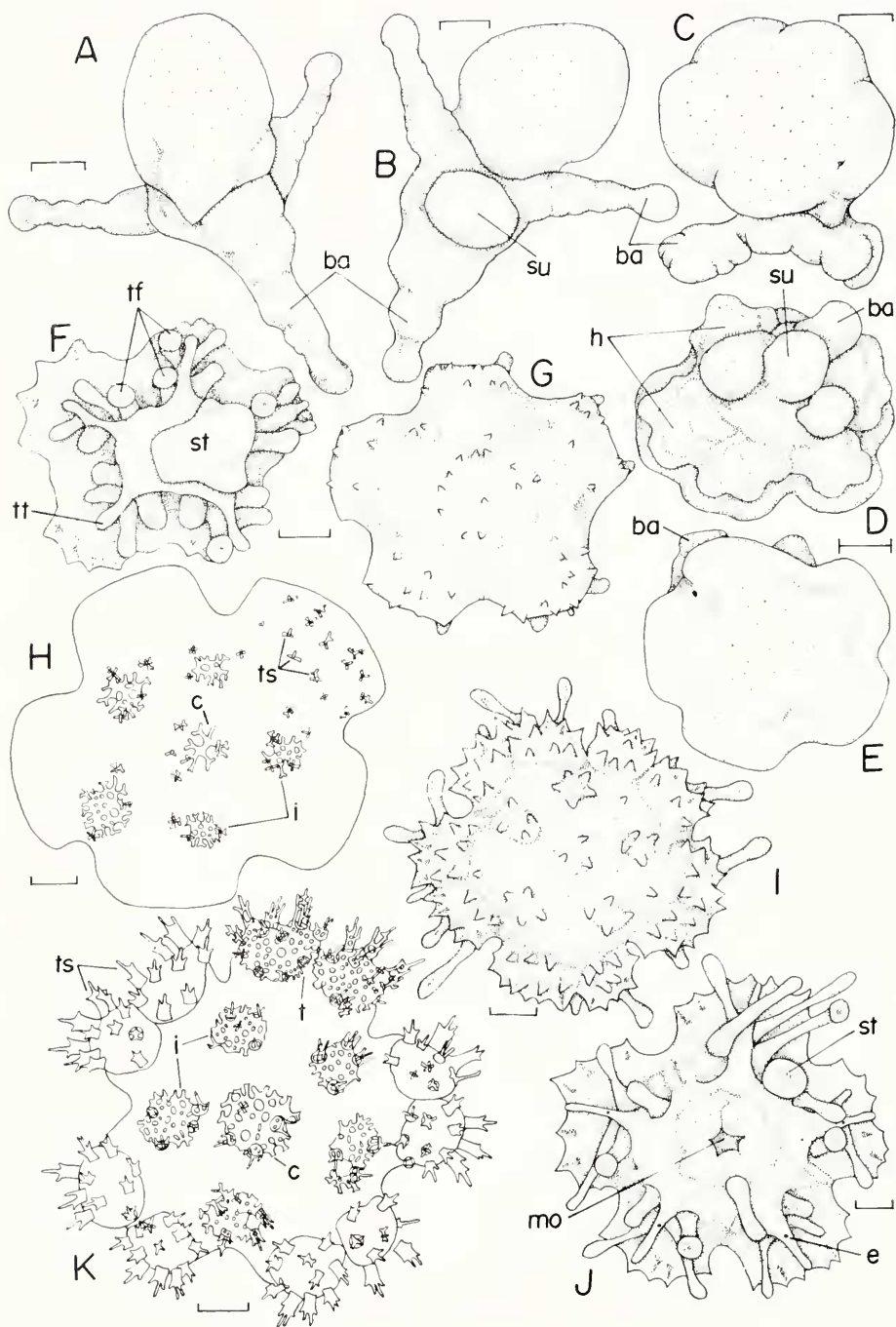
FIGURE 6. Development of specimens of *Asterina minor*. All drawings are from a living specimen, jelly layer is not shown except in A. All scales show 100  $\mu$ m. A) Two-cell stage. f, fertilization membrane; j, jelly layer. B) Eight-cell stage, 4.5 hr after spawning. f, fertilization membrane. C) 64-cell stage, 6.5 hr after spawning. bm, blastomere. D) Early wrinkled blastula, 10 hr after spawning. cm, cell mass; et, egression tract. E) The most wrinkled blastula, 12.5 hr after spawning. F) Early gastrula, 18 hr after spawning. Egression tracts (et) are still recognized on the surface of the embryo, view from the vegetal pole. bp, blastopore. G) Early gastrula, 18 hr after spawning, view from the animal pole. et, egression tract. H) Gastrula, 36 hr after spawning. I) Gastrula, 40 hr after spawning. bp, blastopore. J) Brachiolaria, 2 and a half days after spawning, view from the ventro-lateral (right) side. K) Brachiolaria, 3 days after spawning, ventral view. ba, brachiolar arm; su, sucker. L) Brachiolaria at the commencement of hatching, 4 days after spawning, view from the ventro-lateral (left) side. f, fertilization membrane. M) Brachiolaria, just after hatching, ventral view. ba, brachiolar arm; su, sucker. D) Same as M, dorsal view. ba, brachiolar arm; hp, hydropore. O) Same as M, ventro-lateral (right) view. hp, hydropore.

to the first. The cleavage is total, equal, and radial. The embryo reaches the eight-cell stage (Fig. 6B) and the 64-cell stage (Fig. 6C), 4.5 and 6.5 hr respectively after spawning. Eight hours after spawning, the embryos enter into the wrinkled blastula stage. The process of wrinkling has been earlier reported in detail (Komatsu, 1976). Figures 6D and 6E illustrate an early wrinkled blastula and most wrinkled blastula, respectively. Twenty-four hours after spawning, the wrinkled blastula stage is completed.

At the end of the wrinkled blastula stage, gastrulation by invagination takes place (Figs. 6F, 6G). Thirty-six hours after spawning, the gastrula begins to rotate within the fertilization membrane (Fig. 6H) and becomes enlarged along the archenteric axis. Figure 6I shows a gastrula 40 hr after spawning. It measures about 500  $\mu\text{m}$  in length and 350  $\mu\text{m}$  in width. Two days after spawning, the ventral side of the embryo becomes flattened and the rudiments of the brachiolar arms (lateral arms) appear at the ventro-lateral side of the embryo. These arms grow gradually and the third arm (anterior arm) emerges from the ventral side of the anterior portion of the embryo. Two and a half days after spawning, the embryo becomes pear shaped, with three distinct brachiolar arms and a rudimentary sucker which appears in the area surrounded by the brachiolar arms (Fig. 6J). Three days after spawning, the brachiolaria measures 550  $\mu\text{m}$  in total length (Fig. 6K). The anterior half of the embryo, which bears three brachiolar arms and a sucker, corresponds to the stalk of the larva and the posterior half is the disk of the larva. Lateral arms are longer, 150  $\mu\text{m}$  in length, than the anterior arm, which is about 100  $\mu\text{m}$ . A blastopore and a hydropore are observed at the posterior tip and at the dorsolateral (right) side of the larva, respectively. Three and a half days after spawning, the blastopore is closed.

About four days after spawning, the brachiolaria is hatched from the fertilization membrane (Figs. 5I, 5L), usually at the anterior portion. Figures 6M, 6N and 6O show a freshly hatched larva. The present species has no pelagic life and the brachiolariae creep on the substratum with their developed brachiolar arms throughout the brachiolaria stage (Fig. 5J). One day after hatching, the brachiolariae attach to the substratum with their brachiolar arms and not with the sucker (Figs. 7A, 7B). This attachment is a sign of the commencement of metamorphosis. At this stage, the brachiolar arms are markedly long and appear semi-transparent excepting at the tip ends. The anterior arm measures 250  $\mu\text{m}$ , and the lateral arms are 350  $\mu\text{m}$  in length.

About 12 hr later, the disk begins to transform into a subpentagonal form (Fig. 7C), and the stalk begins to shrink rapidly. The rudiments of the tube-feet are seen on the hydrolobes, these are clearly visible in the future oral side of the adult disk. Figures 7D and 7E show a larva one day after the one shown in Fig. 7C. This larva is about 500  $\mu\text{m}$  in diameter and has a hydropore in an inter-radius of the future aboral side of the disk. A degeneration of the stalk, including the brachiolar arms and sucker, progresses gradually. About 7 days after spawning, the larvae are freed from the substratum due to the extreme degeneration of the brachiolar arms. They are able to move by means of their tube-feet (Figs. 7F, 7G). On the aboral side of the larva at this stage, one central and five inter-radial plates are recognized (Fig. 7H). Metamorphosis is completed with the opening of the mouth about 10 days after spawning (Figs. 7I, 7J). At this stage,



the juvenile is 700  $\mu\text{m}$  in diameter, and bears a red eye-spot at the basal portion of each terminal tentacle. On the oral side, a rudiment of the stalk is still observed. In each interradius, the formation of a pair of oral plates is noticed. Skeletal plates on the aboral side are shown in Fig. 7K. It is of interest to note that a slit is seen at the midline of each terminal plate, making it appear as if one terminal plate is composed of two pieces. Fifteen days after spawning, that is about 5 days after the completion of metamorphosis, the juveniles start moving from the place where they were laid (Figs. 5K, 8A). At this stage, each oral plate bears a spine which is pointed at the center of the mouth (Figs. 8B, 8C). On the aboral side of disk, small radial plates are being formed (Fig. 8D). After the spawning the juveniles grow to the size of 1000 and 1200  $\mu\text{m}$  in diameter after 20 and 30 days, respectively. In specimens 30 days after spawning, each radial plate bears one spine at the center. In each interradius of the aboral side, one pair of the secondary plates is formed. On the oral side, a pair of adambulacral plates is formed in each arm and each adambulacral plate is furnished with one spine.

The juveniles were kept in the laboratory several months after the completion of metamorphosis, but no further development was observed beyond the stage 30 days after spawning (20 days after the completion of metamorphosis). Field surveys were then undertaken to obtain supplementary material for understanding post-metamorphic development. As shown in Table II, a number of juveniles were collected. Among these, two animals are described here in detail. One animal collected on July 3, 1974, was 1500  $\mu\text{m}$  in diameter and had three pairs of the tube-feet in each arm (Figs. 9, 10, 11A). At each interradius, one pair of superomarginal plates was present, each plate having two prominent spines. On the oral side, five pairs of oral plates encircle the mouth. In each arm, two pairs of adambulacral plates and one pair of inferomarginal plates were present. The first adambulacral plate had two small spines. The second adambulacral plate was smaller than the first, and each of them had one spine. The other specimen collected on Sept. 7, 1973, was 3000  $\mu\text{m}$  in diameter and possessed seven pairs of tube-feet in each arm (Figs. 11B, 11C). This specimen had well-developed aboral skeletal plates, some of which were imbricated with each other. Between the neighboring interradiial plates, there was papular area, each of which had one papula. In one of the interradiial plates there was a concave portion and this might be a rudiment of the madreporite. Each arm had several pairs of supero-

FIGURE 7. Development of specimens of *Asterina minor*. All drawings are made from living specimens, except H and K, which were treated with KOH solution before examination. All scales show 100  $\mu\text{m}$ . A) Brachiolaria, 5 days after spawning, strongly attached to substratum with brachiolar arms (ba), dorsal view. B) Same as A, ventral view. ba, brachiolar arm; su, sucker. C) Metamorphosing larva, 12 hr after that shown in Figures 7A and B, view from the future aboral side of the juvenile. ba, brachiolar arm D) Metamorphosing larva, 1 day after that shown in Figure 7C, future oral view. ba, brachiolar arm; h, hydrolobe; su, sucker. E) Same as D, future aboral view. ba, brachiolar arm. F) Metamorphosing larva, 7 days after spawning, future oral view. st, stalk; tf, tube-foot; tt, terminal tentacle. G) Same as F, future aboral view. H) Aboral skeletal plates, in the same stage shown in Figures 7F and G. c, central plate; i, interradiial plate; ts, spines on the terminal plate. I) Juvenile just after the completion of metamorphosis, 10 days after spawning, aboral view. J) Same as I, oral view. e, eye-spot; mo, mouth; st, markedly degenerated stalk. K) Aboral skeletal plates, in the same stage shown in Figures 7I and J. c, central plate; i, interradiial plate; t, terminal plate; ts, spines on the terminal plate.



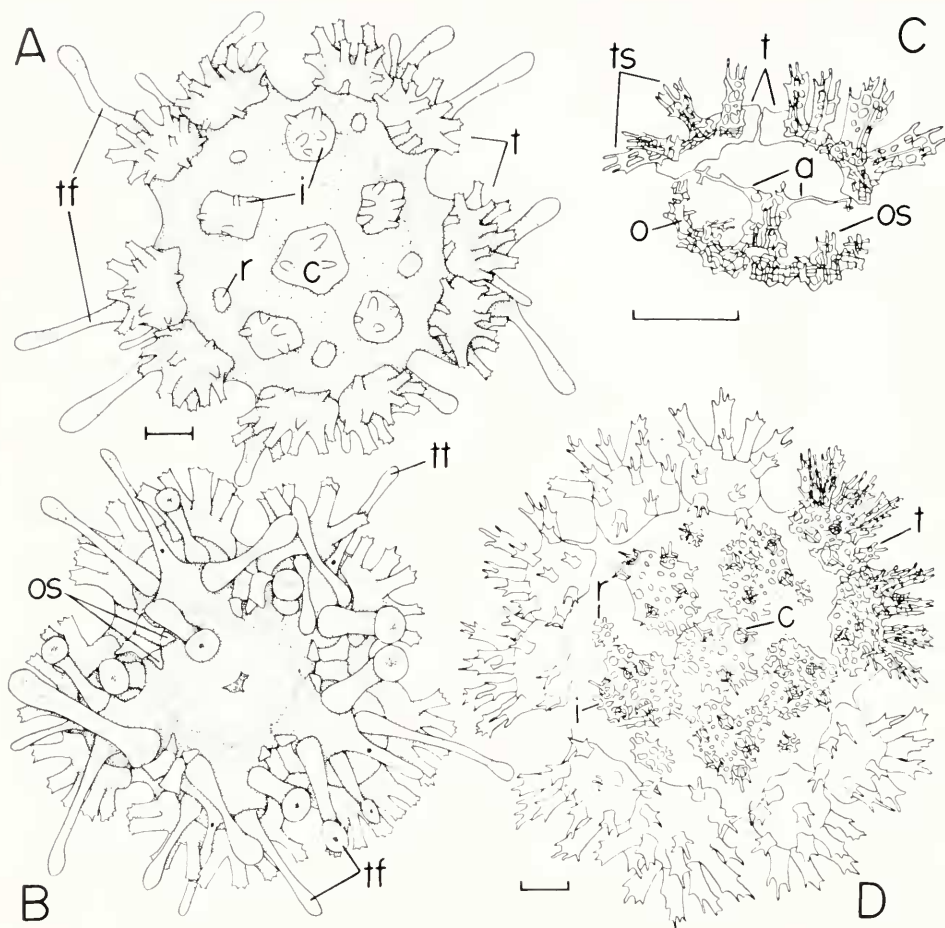


FIGURE 8. Juvenile of specimens of *Asterina minor*, five days after the completion of metamorphosis, having two pairs of tube-feet. A and B are drawn on living specimens and C and D are on KOH treated specimens. All scales show 100  $\mu\text{m}$ . A) Aboral view. c, central plate; i, interradial plate; r, radial plate; t, terminal plate; tf, tube-foot. B) Oral view. os, oral spine; tf, tube-foot; tt, terminal tentacle. C) Skeletal plates of an arm, oral view. a, ambulacral plate; o, oral plate; os, oral spine; t, terminal plate; ts, spine on the terminal plate. D) Aboral skeletal plates. c, central plate; i, interradial plate; r, radial plate; t, terminal plate.

marginal plates and inferomarginal plates, the latter both in size and number were larger than the former. Six pairs of adambulacral plates were present in each arm, and each of the three proximal plates (1st, 2nd, and 3rd) possessed three spines. In the interradial area of the oral side, there were several ventro-lateral plates in addition to adambulacral plates and inferomarginal plates. One slit was always recognizable along the midline of the terminal plate.



FIGURE 9. Aboral skeletal plates of a juvenile specimen of *Asterina minor*, with three pairs of tube-feet in each arm, KOH treated. c, central plate; i, interradial plate; r, radial plate; sm, superomarginal plate. Scale = 250  $\mu$ m.

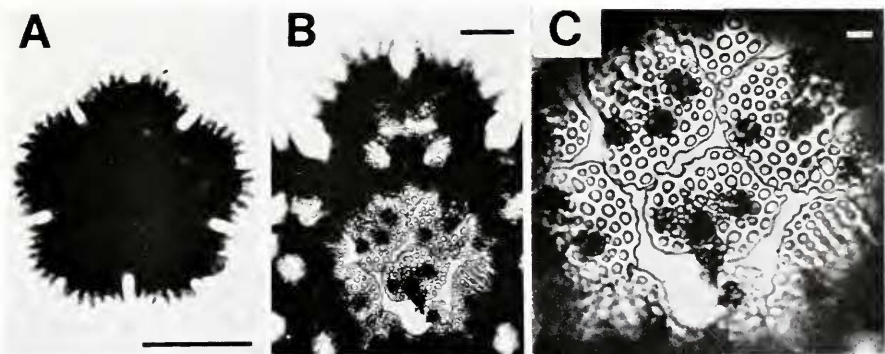


FIGURE 10. Skeletal plates of a juvenile specimen of *Asterina minor*, in the same stage shown in Figure 9, KOH treated. A) Shadow picture showing spines on terminal plates and contour of the body. Scale = 500  $\mu$ m. B) Picture of plates in one arm and disk, in the same specimen shown in Figure A. Shaded area corresponds to plates in the oral side. Scale = 150  $\mu$ m. C) Magnified picture of the central portion of Figure B. Scale = 150  $\mu$ m.

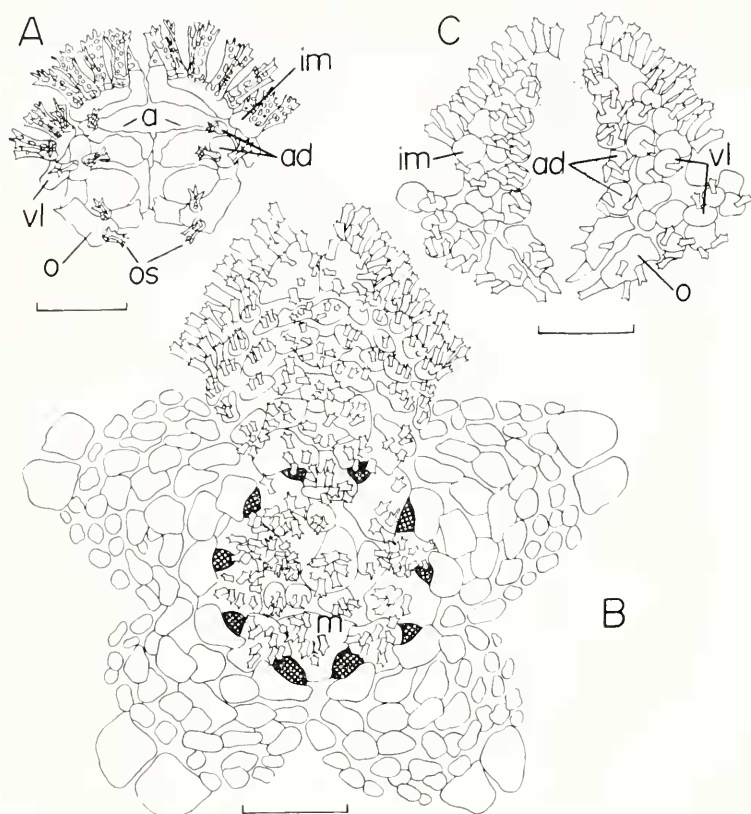


FIGURE 11. Skeletal plates of juvenile specimens of *Asterina minor*, treated with KOH. A) Oral skeletal plates in one arm in the specimen shown in Figure 9. a, ambulacral plate; ad, adambulacral plate; im, inferomarginal plate; o, oral plate; os, oral spine; vl, ventro-lateral plate. Scale = 200  $\mu\text{m}$ . B) Aboral skeletal plates of a specimen having seven pairs of tube-feet in each arm, hatched portions show papular area. m, interradianal plate with a rudiment of madreporite. Scale = 450  $\mu\text{m}$ . C) Skeletal plates in the oral side of one arm in the specimen shown in Figure B. ad, adambulacral plate; im, inferomarginal plate; o, oral plate; vl, ventro-lateral plate. Scale = 500  $\mu\text{m}$ .

### DISCUSSION

*A. minor* lacks the bipinnaria stage and develops without pelagic life as known in *A. gibbosa* and *A. exigua* (Ludwig, 1882; MacBride, 1896; Mortensen, 1921). In general, asteroids whose development is direct have pear-shaped brachiolaria with three brachiolar arms. In species belonging to *Asterina*, the brachiolar arms of *A. batheri* and *A. coronata japonica* are short and blunt (Komatsu, 1975; Kano and Komatsu, 1978). The brachiolariae of these species have pelagic life. On the other hand, the brachiolar arms of *A. minor* are well developed and resemble those of *A. gibbosa* and *A. exigua*, and yet these brachiolariae spend benthonic life creeping on the substratum before metamorphosis. It is likely, therefore, that well-developed brachiolar arms are one of the adaptive characters of the benthonic life.

As described before, asteroids, barring a few hermaphroditic species, are gonochoric (Delavault, 1966). Sexuality of asterinids has been fairly well studied since Cuénot (1887) reported the occurrence of protandric hermaphroditism in *A. gibbosa*. Among asterinid species, in addition to *A. gibbosa*, hermaphroditism is known in *A. pancerii*, *A. batheri* and *A. scobinata* (Ohshima, 1929; Cognetti, 1954; Dartnall, 1970; Kano and Komatsu, 1978). In *A. batheri* a few hermaphroditic individuals occur among gonochoric individuals (Ohshima, 1929). No difference seems to exist in the sexual status of this species from different geographical regions (Kano and Komatsu, 1978). In this study, it was found that *A. minor* is a spatial hermaphroditic and all individuals bear hermaphroditic gonads comprising both functional testes and ovaries. In the breeding season, both elements mature simultaneously and the eggs can be fertilized with the sperm released from the same individual. This study has presented for the first time evidence of definite self-fertilization in asteroids.

Chia (1968) reported that *Leptasterias hexactis* aggregates under rocks during breeding season. Such a gathering is usual behavior in brooding species (Kubo, 1951). It is interesting to note that *A. minor* shows a distinct breeding assemblage, although it is not a brooding species and is capable of self-fertilizing. These facts may imply a complex historical background through which *A. minor* has been speciated.

The data accumulated so far indicate that genus *Asterina*, or its closely related groups, despite intimate similarity in adult morphology in different species, shows a remarkable variety in the mode of development and the method of breeding (Ludwig, 1882; MacBride, 1896; Goto, 1898; Mortensen, 1921; James, 1972; Komatsu, 1975; Kano and Komatsu, 1978). This observation may bring out the importance of studies on the development of *Asterina* group which may not only clarify the ontogeny of a given species but may also help towards understanding the evolution of asteroid development as a typical group having divergent breeding methods and development.

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#### SUMMARY

1. The breeding season of *Asterina minor* is estimated to be during the month of May in Kushimoto, Japan. *A. minor* shows a characteristic breeding assemblage and the eggs are laid on the substratum in a mass spawning. The eggs are not protected by the adults.

2. *A. minor* is a spatial hermaphrodite, where ovaries and testes in an individual become mature simultaneously. Isolated individuals are capable of self-fertilizing and the self-fertilized eggs develop normally.



3. The spawned eggs are spherical, yellow, and  $437\ \mu\text{m}$  in average diameter. They attach to the substratum with a sticky jelly layer. Cleavage is total and radial.

4. Eggs through the wrinkled blastula stage develop into a pear-shaped brachiolaria bearing three brachiolar arms within the fertilization membrane.

5. About four days after spawning, the brachiolariae hatch from the fertilization membrane and creep on the substratum with well-developed brachiolar arms. There is no evidence of pelagic life in the present species.

6. One day after hatching, brachiolariae attach firmly to the substratum with the brachiolar arm and undergo a rapid transformation of the body (metamorphic climax). Metamorphosis is completed with the opening of the mouth about 10 days after spawning. The newly metamorphosed juvenile is about  $700\ \mu\text{m}$  in diameter and each arm bears two pairs of the tube-feet and one red eye-spot at the base of the terminal tentacle.

7. The reproduction and larval development of *A. minor* are unique, and the study may prove a good guideline for understanding the evolution of reproduction and development in Asteroidea.

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